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Original Article

Core classification of head and neck squamous cell carcinomas: Correlations between morphology, DNA ploidy and HPV infection

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ABSTRACT

Aims: The study intended to reveal whether HPV infection is reflected by nuclear morphology and DNA cytometry parameters in head and neck squamous cell carcinomas (HNSCC).
Methods: In total, 39 HNSCC were selected for reanalysis by histomorphology applying the core classification, DNA cytometry and HPV detection. For the core classification, HE sections were assessed by a score system to evaluate the nuclear size, the mitosis size, their variabilities and the presence of tripolar or tetrapolar mitoses. HPV was analyzed by consensus PCR followed by a hybridization method for virus typing. Static DNA cytometry was applied on single cell suspension focusing particularly on the parameters DNA modal value, DNA index peak, DNA index mean, 2c deviation index and 5c exceeding rate. Statistical analysis was done by *T*-test or Fisher's exact test.
Results: The analysis revealed that HPV positive HNSCC had significantly smaller nuclei than HPV negative cases. Increasing values of the nuclear size and mitosis size were significantly associated with higher indices of the DNA cytometry analysis.
Conclusions: The study confirms that the core classification can provide information on the ploidy of HNSCC and that HPV positive tumors represent a distinct morphological and genetic carcinoma subtype.

Introduction

Among malignancies worldwide head and neck cancer ranks sixth. Dependent on tumor sites incidence rates vary from 0.9 to 11.2/100,000 for males and from 0.3 to 4.3/100,000 for females, and show preference for the developed countries' nasopharynx carcinomas excepted (GLOBALCAN 2002. http://www-dep.iarc.fr./). Despite advances in treatment there has been no significant decline in mortality rates yet. For a subset of head and neck squamous cell carcinomas (HNSCC), approximately 20% of the tumors, there is evidence that human papillomavirus (HPV) plays an etiologic role in the development of malignancy and subtype 16 is the most important among high risk human papilloma viruses to be found in these specimens. HPV positive tumors occur at any sites of the head and neck, but especially carcinomas in the oropharynx, at the same time the most common tumor site, seem to be associated with HPV infections, particularly palatine tonsil cancers [7,13,28].

Earlier studies describe differences in behavior and prognosis, etiological aspects, histomorphology and immunohistochemistry profiles which suggest HPV positive cancers to be a distinct tumor type compared to HPV negative tumors of the head and neck [4,5,6,9,13,15,17,19,23,25–27]. In our study we analyzed cancer specimens of the head and neck using a recently developed core classification scheme [20] which allowed to detect differences between HPV positive and negative HNSCC. Furthermore, we examined whether these distinctions are reflected by DNA cytometry parameters.

Methods

Tumor collective

Our tumor collective comprised 46 head and neck squamous cell carcinomas of different sites. 22 cases were carcinomas of the oropharynx, thereof 16 carcinomas of the palatine tonsil. Fur-

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Table 1
Core classification: parameters of the morphological analysis.

Category	Description	Score
Core size[a,b]		
Small	Nuclear size/volume ≤3 lymphocytes	1
Medium	Nuclear size between 4 and 6 lymphocytes	2
Large	Nuclear size between 7 and 9 lymphocytes	3
Giant	Nuclear size ≥10 lymphocytes	4
Mitosis size[a,b]		
Small	Mitosis size ∼1–1.5 lymphocytes	1
Medium	Mitosis size between 1.5 and 2 lymphocytes	2
Large	Mitosis size ≥2 lymphocytes	3
Core/mitosis size variability		
Absent	Only one score	0
Little	Two scores present	1
Strong	Three scores	2
Strong plus giant cells	Four scores present	3
Tripolar/tetrapolar mitosis[a]		
Absent	Not detectable	0
Present	At least one mitosis found	1
Frequent	Mitoses easily found	2

The core classification provides a less subjective evaluation of nuclear histomorphology by means of internal reference cells.
[a] One representative tumor section was analyzed.
[b] For the nuclear size and mitosis size all score values are listed in descending order of their relative frequency.

thermore we examined 11 larynx carcinomas, 8 hypopharynx carcinomas, 2 carcinomas of the oral cavity and 3 lymph node metastases.

HPV detection and typing

For detection of HPV in tissue samples, PCR analysis was performed using consensus primers for the amplification of the L1-region as described previously [7,12].

To identify HPV subtypes, samples also underwent hybridization analysis, using specially designed microchips (Chipron GmbH, Berlin) with DNA probes of the major high and low risk HPV types. The LCD-array HPV 3.5C contained primer pairs and capture probes specific for the HPV low risk types 06, 11, 42, 43, 44 and the high risk types 16, 18, 31, 33, 35, 39, 45, 51, 52, 55, 56, 58, 59 and 68. Hybridization procedures were performed according to the manufacturer's protocol.

Morphological analysis

H&E sections of all cancer samples were reviewed to classify the tumors according to specific histomorphological criteria. The nuclear size, the mitosis size and their variability were assessed by using a scoring system in which lymphocytes served as internal reference (Table 1).

DNA measurement

DNA measurement could be performed on 33 tumors specimens on Feulgen stained single cell suspensions by static DNA cytometry [3] using a standard light microscope (Leitz DMRB, Leica, Germany) with 40× objective connected via a calibrated color camera (Sony 3CCD-Iris-Model-DXC-930P; Japan) to a monitor and a personal computer equipped with the AutoCyto QUIC-DNA software system (TriPath Imaging Burlington, NC, USA) as previously described [20]. The DNA measurements provided the following parameters [10,11]:

- Stemline indices: DNA modal value, DNA index mean, DNA index peak.
- Basic indices: 2c deviation index (2cDI).
- Relative indices: 5c exceeding rate (5cER).

Statistical analysis

SPSS software (version 13, SPSS GmbH Software, Munich, Germany) was used for statistical analysis. p-Values smaller than 0.05 were considered as statistically significant, $p < 0.005$ as highly significant. The means of the parameters nuclear size, mitosis size, core and mitosis size variabilities between HPV negative and positive subgroups were established by Fisher's exact test. Means of DNA cytometry parameters for both subgroups were investigated by T-test. Correlations of the means between DNA cytometry and histomorphology parameters were investigated by the Anova test.

Results

Detected HPV infections

HPV infection was detected in 20 HNSCC, mostly subtype 16. Subtype 33 was found in a lymph node metastasis and subtype 35 in an oropharynx carcinoma. Furthermore one mixed infection of HPV 16 and 35 occurred in a palatine tonsil carcinomas.

Core classification of Head and neck cancers

In histomorphological analysis our core classification revealed HPV positive cancers to have on average significant smaller nuclei than HPV negative tumors (1.29 versus 1.62, $p = 0.019$). However, no significant differences between HPV positive and negative neoplasias could be detected for mitosis sizes and core/mitosis size variabilities.

Correlations between histomorphology and DNA cytometry parameters

In general, for all tumors, there was a highly significant correlation between nuclear/mitosis size respectively and DNA modal value, DNA index mean and 2cDI ($p = 0.001$). Furthermore we found significant correlations between nuclear/mitosis size and 5cER ($p = 0.03/p = 0.002$), and a significant correlation between mitosis size variability and 2cDI ($p = 0.043$).

Correlations between HPV status and DNA cytometry parameters

In DNA cytometry HPV positive tumors showed significantly lower DNA modal values (2.32 versus 2.91; $p = 0.039$) and DNA index means (2.51 versus 3.16; $p = 0.011$) than negative cancers. The DNA index peak was also lower for HPV positive tumor, but statistically without significance. Furthermore, lower 2cDIs and 5cERs in DNA cytometric analysis of HPV positive specimens were highly significant (1.10 versus 2.72; $p = 0.009$ and 1.32 versus 7.74; $p = 0.004$).